for the amendments to claims 8, 12-15, 17, 18, 21, and 36 with respect to the functional activity of *ced-3* related nucleic acids, can be found at page 13, lines 20-25 which state,

> "Functionally related genes refer to genes which have similar activity to that of *ced-3* or *ced-4* in that they cause cell death. Such genes can be identified by their ability to complement *ced-3* or *ced-4* mutations in bioassays, as described below."

Such *in vivo* and *in vitro* bioassays are generally described in the specification at page 17, line 15 to page 20, line 4. More specifically, an *in vivo* complementation assay, where *ced-3*(+) DNA capable of complementing the *ced-3* mutant phenotype is identified, is described in Example 2, particularly at page 52, line 11 to page 54, line 24. Support for the amendments to claims 17, 18, and 21, with respect to the structural characteristics of *ced-3*, can be found, for example, at page 11, lines 24-34.

We note here that amendment of claim 13 part (e), where position 417 is deleted and position 412 is added, is supported in Table 3 where position 412 of the *ced-3* gene is the indicated position for this mutation. The original entrance of 417 into claim 13 is believed to be a typographical error. Applicants apologize for this oversight.

REMARKS

Summary of the Office Action

The invention features wild-type and mutant *ced-3* genes and structural and functional equivalents thereof. The *ced-3* gene is required for the onset of programmed

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cell death in *C. elegans*. Such genes that increase or decrease cell death are useful, for example, in the treatment of disorders and conditions characterized by aberrant increases or decreases in cell death, including cancer, neural and muscular degenerative diseases, stroke, traumatic brain injury, myocardial infarction, viral (e.g., HIV) and other types of pathogenic infections, as well as cell death associated with normal aging and hair loss.

Claims 1-4, 8-15, 17, 18, 21, 22, 25-27, 33, 35, 36, and 40 were examined in this case. All claims stand rejected. The present response cancels claims 9, 10, 11, 22, 25-27, 33, 35, and 40 and amends claims 1-4, 8, 12-15, 17, 18, 21, and 36. Each of the objections and rejections levied in the Office Action is addressed individually below.

Election

The Examiner acknowledged Applicants amendment of June 17, 1999 (Paper No 10), electing the invention of group I. Applicants note that although claims 25-27, 33, 35, and 40 were included in this restriction group, as noted by the Examiner on page 6 of the Office Action, these claims depend on non-elected claims and have been canceled by the present amendment. Applicants reserve the right to prosecute these claims in a future application.

Priority

Amendment of the specification is made in response to the Examiner's suggestion

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that for receiving the benefit of an earlier filing date under 35 U.S.C. §119(e), the application must contain specific reference to the prior applications in the first sentence of the specification (37 CFR 1.78). Accordingly, the first sentence of the application now correctly claims priority from all earlier applications. Withdrawal of this objection is requested.

Rejections Under 35 U.S.C. §112, First Paragraph

Claims 1-4, 8-15, 17, 18, 21, 22, 33, 35, 36, and 40 stand rejected for lack of enablement. The Examiner acknowledges that the specification is enabling for: the *ced-3* gene disclosed in SEQ ID NO:18; the DNA encoding the amino acid sequence of SEQ ID NO:19; the *ced-3* mutants listed in Table 3, page 62, of the specification; and the *ced-4* mutants listed in Table 2, page 61, of the specification. However, the Examiner states that the specification fails to reasonably provide enablement for isolated DNAs for any and all *ced-3* genes, the RNA encoded thereby, isolated DNAs for any and all *ced-3* or *ced-4* genes, or probes thereof. Applicants have canceled claims to *ced-4* and amended the scope of the remaining claims.

Specifically, claims 1-4 have been amended to add specific structural and functional limitations to clearly differentiate the *ced-3* genes of the invention from "any an all" *ced-3* genes, as stated by the Examiner. Claims 8, 12-15, and 17-18, which pertain to new and mutant *ced-3* genes, have been similarly amended to provide a means to

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identify a mutant *ced-3* gene by its activity. Claim 21, directed to probes and primers, has also been amended to recite structural and functional limitations, as well as specific nucleic acid sequences of the *ced-3* gene. Claim 36 has been amended to be independent and to further define mutants of the *ced-3* gene.

ced-3 Gene Family Members

The present invention demonstrates the identification of a cell death gene family. In particular, the specification demonstrates identification of additional family members based on the structural and functional parameters provided for this gene family in the specification. One aspect of this teaching is that these characteristics are applicable to the identification of cell death genes in other organisms (e.g., mammals). Those of ordinary skill in the art would recognize that additional cell death genes may be routinely isolated from other organisms using the structural and functional characteristics provided by the specification for this gene family. Another aspect of this teaching is that these characteristics are similarly applicable to the identification of mutant cell death genes, as described in the specification.

The specification teaches a repeatable process by which cell death genes and mutants thereof are identified. In order to achieve this, the specification teaches that one 1) isolate a nucleic acid encoding at least a portion of a cell death gene or cell death gene mutant based on the structural characteristics of cell death genes; and 2) test the function

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of the putative cell death gene or cell death gene mutant in an *in vivo* or *in vitro* bioassay.

Isolation of Cell Death Genes

In order to carry out steps 1) and 2) set forth above, one could, of course, rely on the structural and functional characteristics provided in the specification for the claimed *ced-3* cell death genes. Initially, the specification describes the isolation of *ced-3*. The structure of this gene is described in detail in the specification. For example, the *ced-3* gene is highly hydrophilic. In the description of *ced-3* provided in the specification, "very hydrophilic" is defined as follows:

> "The protein is very hydrophilic and no significantly hydrophobic region can be found that might be a transmembrane domain" (page 11, lines 25-28).

In addition, the *ced-3* gene has further defining structural characteristics. Specifically, the *ced-3* gene has a serine rich region that is sensitive to mutation and is conserved among other *ced-3* genes of different homologs (page 11, line 28 to page 12, line 9).

These specific structural parameters, in combination, provide the skilled artisan with sufficient information to design probes and primers useful for cloning additional cell death genes using methods standard in the art. As taught by the specification, "[g]enes which are structurally related to *ced-3* [...] are likely to also act as cell death genes" (page 15, lines 16-17). The specification goes on to teach that

> "[s]tructurally related genes can be identified by any number

> of detection methods which utilize a defined nucleotide or amino acid sequence or antibodies as probes. For example, nucleic acid (DNA or RNA) containing all or part of the *ced-3* [...] gene can be used as hybridization probes or as polymerase chain reaction (PCR) primers. Degenerate oligonucleotides derived from the amino acid sequence of the Ced-3 [...] proteins can also be used. Nucleic acid probes can also be based on the consensus sequences of conserved regions of genes or their protein products. In addition, antibodies, both polyclonal and monoclonal, can be raised against the Ced-3 [...] and used as immunoprobes to screen expression libraries of genes."

In light of the teachings of the specification, the present invention clearly provides the skilled artisan with guidance on how to isolate genes and gene mutants based on the structural characteristics of *ced-3* identified by the present invention.

In further support of this assertion, the specification further provides a specific strategy for detecting structurally related genes in other organisms and demonstrates the successful completion of the first step in that strategy. Specifically, the specification teaches that "one strategy for detecting structurally related genes in other organisms is to initially probe animals which are taxonomically closely related to the source of the probes, for example, probing other worms with a *ced-3* [...] probe" (page 15, line 33 to page 16, line 2). The specification goes on to teach that "sequences conserved between *ced-3* [...] and these new genes can then be used to identify similar genes from less closely related species" (page 16, lines 5-7).

Indeed, the specification demonstrates the cloning of two additional *ced-3* genes

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from other *Ceanorhabditis* species, *C. briggsae* and *C. vulgaris*. The specification demonstrates that "serine rich regions were found in the polypeptides encoded by all three genes" (see, page 16, lines 29-30 and Example 2). With the sequence of multiple *ced-3* genes in hand, the skilled artisan can apply steps 1 and 2 set forth above to identify additional cell death genes and cell death gene mutations. The specification provides explicit guidance for execution of steps 1 and 2 set forth above, as described in further detail below.

The *ced-3* genes identified by the specification classify as a gene family, or a group of structurally related genes (as defined by the specification at page 16, lines 17-19). The specification further points out that "comparison of members within a gene family, or their encoded product, may indicate functionally important features or the genes or their gene products. Those features which are conserved are likely to be significant for activity. Such conserved sequences can then be used to identify new members of the gene family" (page 16, lines 19-25). The *ced-3* gene meets this criteria. First, the *ced-3* genes share a conserved serine rich region. Second, the conservation of the number of serines in the serine rich region suggests that the serine rich feature is important for function (page 16, lines 29-35).

Function of Cell Death Genes

With respect to step 2), testing the function of a putative cell death gene or cell

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death gene mutant, the specification defines "functionally related genes" as those "which have similar activity to that of *ced-3* or *ced-4* in that they cause cell death" (page 13, lines 21-22). The specification proceeds to teach that "such genes can be identified by their ability to complement *ced-3* or *ced-4* mutations in bioassays" (page 13, lines 22-25). Specific *in vivo* and *in vitro* bioassays are described at page 17, line 15 to page 20, line 4. A working example of one such bioassay, an *in vivo* rescue assay, is provided in Example 2, page 52, line 11 to page 54, line 24. Thus, the specification not only clearly defines the meaning of functional activity, but also explicitly provides a means for assaying for the activity and demonstrates successful use of the assay to verify the function of a cell death gene.

As illustrated above, the skilled artisan could easily follow the teachings of the present invention to isolate additional cell death genes from other organisms. Alternatively, the skilled artisan could follow the teachings of the present invention to identify additional mutant cell death genes. The specification teaches that mutational analysis can be carried out on the *ced-3* gene and even provides guidance for making mutations in the *ced-3* gene (see, page 17, lines 6-14). The mutant cell death genes, according to the present invention, can then be tested for their ability, or lack thereof, to complement *ced-3* or *ced-4* mutations in a bioassay (see, page 17, lines 17-26 and generally page 17, line 15 to page 20, line 4). Indeed, the testing of a *ced-3* mutant is demonstrated by the present invention at page 53, line 18 to page 54, line 24. In light of

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these teachings, it would be straightforward for the skilled artisan to 1) isolate a nucleic acid encoding at least a portion of a cell death or a mutant cell death gene; and 2) test the function of the putative cell death gene or mutant cell death gene in an *in vivo* or *in vitro* bioassay.

In Office Action, the Examiner questioned the use of making all of these DNAs in the absence of any function. The Examiner further stated that there would be no way of knowing what sequences would be obtained and what would have their function of use been, if any.

In response, the nucleic acids of claims 1-4, 8, 12-15, 17, 18, 21, and 36 have been amended to more clearly define the structural and/or functional parameters for cell death genes, as described in the specification. Claims 22, 33, 35, and 40 have been canceled, rendering the present rejection to these claims moot. The claims now recite specific structural characteristics which must be retained by the nucleic acid and/or further recite a specific function (cell death activity) for the nucleic that is measurable by the ability of the nucleic acid to complement *ced-3* or *ced-4* mutations in an *in vivo* or *in vitro* bioassay.

Summary

In summary, the specification provides sufficient guidance and working examples so that no undue experimentation is required to identify additional family members or mutants of cell death genes. The present invention provides a detailed description of a

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novel family of cell death genes. Given this information, any skilled artisan would recognize that this finding can be used to identify additional nucleic acids related structurally and functionally to the family of *ced-3* nucleic acids described herein. In light of these teachings, Applicants submit that the specification fully enables claims of the present scope, as amended herein.

Rejections Under 35 U.S.C. §112, First Paragraph

Claims 1, 4, 8, 15, 17, 18, 22, 33, 35, 36, and 40 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonable convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. First the Examiner states that a representative number of species have not been described. In particular, the Examiner states that a representative number of species have not been sufficiently described by other relevant identifying characteristics (i.e., other than nucleotide sequence) and there is no disclosure about the extent or type of sequence or functional relationship. Applicants respectfully disagree.

As pointed out above, the claims have been amended to point out the extent or type of sequence or functional relationship that the claimed nucleic acids must have. For example, claims 1, 4, 8, and 15 now recite a *ced-3* nucleic acid sequence that further has the ability to complement *ced-3* or *ced-4* mutations in an *in vivo* or *in vitro* bioassay.

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Claims 17 and 18 also recite this functional limitation and in addition recite specific structural limitations for the *ced-3* nucleic acid. Specifically, claims 17 and 18 recite *ced-3* nucleic acids that encode polypeptides that are hydrophilic in nature and have a serine rich region. Claim 36 has been amended to be independent and to recite ced-3 mutants with a functional limitation. Claims 22, 23, 35, and 40 have been canceled rendering rejection to these claims moot.

Structure and Function of Cell Death Genes

First, the specification provides a detailed description of genes and other nucleic acids that are functionally related to the cell death gene, *ced-3*. As described above, the specification defines "functionally related genes" as those "which have similar activity to that of *ced-3* or *ced-4* in that they cause cell death" (page 13, lines 21-22), and further that "such genes can be identified by their ability to complement *ced-3* or *ced-4* mutations in bioassays" (page 13, lines 22-25). Specific *in vivo* and *in vitro* bioassays that can be used to measure the activity of a cell death gene, according to the invention, are described at page 17, line 15 to page 20, line 4. A detailed, working example of such a bioassay (an *in vivo* rescue experiment), where a nucleic acid fragment is demonstrated to complement the *ced-3* mutant phenotype, is provided by Example 2, at page 52, line 11 to page 54, line 24. In addition, the specification describes in detail a significant number of identifying characteristics, including those which have been incorporated into the claims

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by amendment, that convey that the Applicants were in full possession of the invention, as now claimed. As pointed out above, the *ced-3* gene is highly hydrophilic as defined at page 11, lines 25-28. In addition to being highly hydrophilic, the *ced-3* gene has a serine rich region that is sensitive to mutation and is conserved among other *ced-3* genes of different homologs (page 11, line 28 to page 12, line 9).

Identification of Cell Death Mutants

The Examiner further states that no indication of what other nucleotides would have been mutated in addition to the single nucleotides specifically identified. This assertion is misguided. On page 17, lines 1-14, the specification teaches that "functionally important regions can also be identified by mutagenesis." The specification then proceeds to teach successful use of this strategy. The specification demonstrates that inactivating mutations of *ced-3* were found to cluster within a region near the COOH-terminus (Figure 5B) and suggests that this region is a functionally important domain of the Ced-3 protein. Therefore, the specification demonstrates that mutational analysis can be successfully carried out on the *ced-3* gene. Page 17, lines 1-14 even provide guidance on how to perform *in vivo* and *in vitro* mutagenesis by referring the skilled artisan to a common molecular biological handbook that includes methods of mutagenesis. Based on these teachings, combined with the statement that "those features which are conserved [such as those described above] are likely to be significant for activity" (page 16, lines

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22-24), the skilled artisan would appreciate that it would be a matter of mere routine experimentation to identify additional cell death genes that fit the profile of the cell death genes of claims 1, 4, 8, 15, 17, and 18.

In a related aspect, the specification demonstrates identification of a number of mutants of the *ced-3* gene (see, Example 2 and Table 3). The skilled artisan could simply repeat the methods demonstrated in Example 2 and sequence any EMS-induced *ced-3* alleles in order to identify mutants of the *ced-3* gene.

Number of Species

In response to the Examiner's assertion that a representative number of species have not been sufficiently described by other relevant identifying characteristics (i.e., other than nucleotide sequence) and there is no disclosure about the extent or type of sequence or functional relationship. Applicants point out that the specification demonstrates the cloning of three *ced-3* genes from *C. elegans*, *C. briggsae,* and *C. vulgaris* (page 11, lines line 29 to page 12, line 2). The specification further demonstrates that "serine rich regions were found in the polypeptides encoded by all three genes," suggesting that the serine rich feature is important for the function of *ced-3* (see, page 16, lines 29-35 and Example 2). Moreover, the claims have been amended to recite a specific functional limitation that can be applied to the *ced-3* gene; functionally related genes refer to genes which have similar activity to the *ced-3* gene in that they cause cell death which

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can be measured by their ability to complement *ced-3* or *ced-4* mutations in bioassays, as described above. Thus, Applicants assert that the specification provides a written description sufficient to meet the enablement standard for the claims, as amended herein.

Standard of Perfection

Finally, the Examiner applies a standard of perfection with respect to enablement that finds no basis in the statute or case law. The Examiner requires that every conceivable embodiment falling within the claims perform successfully, with failures in thought experiments in "extreme cases" negating enablement. If this were the standard, generic claims would never be allowable, in any instance in which an Examiner can imagine a single inoperative embodiment. This is not the standard the law requires. For example, in Application of Angstadt, 537 F2.d 498, 190 U.S.P.Q. 218 (C.C.P.A. 1976), the Court, in holding that a claimed invention was enabled though the claim admittedly included inoperative embodiments, stated that "the evidence as a whole, including the inoperative as well as operative examples, negates the PTO position that persons of ordinary skill in this art, given its unpredictability, must engage in undue experimentation to determine which complexes work."

Therefore, in light of the above arguments and amendment of the claims, withdrawal of this rejection is requested.

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Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 1-4, 8-15, 17, 18, 21, 22, 25, 33, 35, 36, and 40 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. This rejection has several aspects, each of which are addressed individually below.

First, the Examiner states that claims 1-4, 8-15, 17, 18, 21, 22, 25, 33, 35, 36, and 40 are indefinite because these claims define the nucleic acids by name only, irrespective of their function and structure. In response, claims 1-4, 8, 12-15, 17, 18, 21, and 36 have been amended to recite the specific nucleic acid sequence identifier and functional limitation. In addition, claims 8-15 stand rejected as being indefinite because they recite "the activity of the gene." Claims 8,12-15 have been amended to eliminate this recitation and replace it with a clear and concise statement that the "activity" is cell death activity which is the ability of said mutated *ced-3* gene to complement *ced-3* or *ced-4* mutations in an *in vivo* or *in vitro* bioassay. Claims 22, 25, 33, 35, and 40 have been canceled, rendering the present rejection of these claims moot. In light of these amendments, withdrawal of the rejection to claims 1-4, 8-15, 17, 18, 21, 22, 25, 33, 35, 36, and 40 is requested.

The Examiner rejects claims 17, 18, 21, and 22 as being indefinite due to the phrases "structurally related" and "functionally related." Furthermore, the Examiner states that in relation the degree of relatedness of a structure or function is not revealed. Claims 17, 18, and 21 have been amended to recite specific structural characteristics of

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the *ced-3* gene. These include reference to a specific sequence (SEQ ID NO:18) and further to a nucleic acid encoding a polypeptide that is hydrophilic and has a serine rich region. In addition, Claims 17, 18 and 21 have been amended to recite a specific functional limitation, that of having cell death activity that can be measured by the ability of the nucleic acid to complement *ced-3* or *ced-4* mutations in an *in vivo* or *in vitro* bioassay. Claim 22 has been canceled by the amendment above. In light of these amendments, withdrawal of the rejection to claims 17, 18, 21, and 22 is requested.

The Examiner states that claim 10 is indefinite because it defines mutations by the amino acid, not by nucleotide sequence. In response, claim 10 has been amended to recite the mutation in the nucleic acid sequence with the resultant amino acid change, as suggested by the Examiner. Withdrawal of the rejection to claim 10 is requested.

The Examiner states that claims 21 and 22 are indefinite because they are not limited to nucleic acid, and so do not reflect applicants election of species. Claim 21 has been amended to recite a nucleic acid. Claim 22 has been canceled by the amendment above, rendering the present rejection to claim 22 moot. Withdrawal of the rejection to claims 21 and 22 is requested.

Finally, the Examiner states that claims 25, 33, 35, 36, and 40 are indefinite because they are dependent on non-elected claims. Claim 36 has been amended so that it is now independent. Claims 25, 33, 35, and 40 have been canceled by the present amendment rendering the present rejection of these claims moot. Withdrawal of this

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rejection is requested.

Rejections under 35 U.S.C. §102(b)

The Examiner states that claims 8, 15, 17, 18, 21, 22, 35, 36, and 40 as being anticipated by Yuan (1990) because Yuan teaches the DNA and transcript for *ced-4* gene and its mutant n1416. The Examiner further states that Yuan also teaches that the *ced-4* gene is related to *ced-3* gene and therefore can be used for making probes in addition to teaching bioassays to isolate *ced-3* and *ced-4* genes.

First, Applicants point out that claim 8 has been amended to eliminate recitation of the *ced-4* gene and now recites only *ced-3* nucleic acids. Claim 15, as amended herein, recites *ced-3* RNAs. Claims 17, 18, 21, and 36, like claim 8, are directed to only *ced-3* nucleic acids. Claims 22, 35, and 40 have been canceled rendering the rejection to these claims moot. Therefore, none of the claims is directed to the *ced-4* nucleic acid sequence or the n1416 mutant of *ced-4* disclosed by Yuan et al. Therefore, Yuan cannot anticipate the claims, as amended herein.

With respect to the Examiner's statement that Yuan also teaches that *ced-4* is related to the *ced-3* gene, Applicants assert that this statement is insufficient to anticipate claims 8, 15, 17, 18, 22, 35, 36, or 40. At page 127, lines 8-10, Yuan merely states that "the activities of two of these genes, *ced-3* and *ced-4*, are required for the onset of essentially all *C. elegans* programmed cell deaths." This statement does not disclose the

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sequence of the *ced-3* gene nor does it provide the sequence of a *ced-3* specific probe.

As to the Examiner's statement that Yuan teaches bioassay to isolated *ced-3* and *ced-4* genes, this statement is misguided. It is well known that phenotypic rescue was a popular method of testing the function of identified nucleic acids at the time of the invention. This method was indeed used to identify the wild-type *ced-4* gene. This does not compensate for the fact that the sequence of *ced-3* claimed in claims 8, 15, 17, and 21 is not disclosed in Yuan et al.

In light of the above, it is clear that Yuan et al. does not provide the sequence of the *ced-3* nucleic acids recited in claims 8, 15, 17, 18, and 21. Thus, Yuan cannot anticipate claims 8, 15, 17, 18, or 21 and withdrawal of the rejection to claims 8, 15, 17, 18, and 21 is requested.

Rejections under 35 U.S.C. §102(b)

Claims 17 and 18 stand rejected as being anticipated by Sumrada et al. The Examiner states that the nucleotide sequence disclosed by Sumrada et al. has 100% best local similarity with SEQ ID NO:18 (*ced-3*) in a region of 19 nucleotides. The Examiner further states that since the term structural similarity is not clearly defined in the specification, it is interpreted as any sequence similarity over any number of nucleotides.

Applicants point out that claims 17 and 18 are directed to the entire *ced-3* gene as a whole. Sequence similarity in a region of only 19 out of 7653 nucleotides of the *ced-3*

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gene of Figure 4 in the completely unrelated CAR1 gene of *S. Cerevisiae,* which does not function as a cell death gene, cannot anticipate claims 17 and 18 because each and every limitation of the claim is not disclosed. Withdrawal of this rejection is requested.

Rejections under 35 U.S.C. § 103

Claims 26 and 27 stand rejected under U.S.C. 35 § 103 as being obvious over Yuan et al. Claims 26 and 27 have been canceled by the present amendment rendering the rejection to claims 26 and 27 moot. Withdrawal of this rejection is requested.

Summary

Applicants would like to thank the Examiner for acknowledging that claims 1-4, 9-14, 25 and 33 are free of prior art. Applicants submit that in light of the above, the claims should now be in condition for allowance. No new matter has been introduced by the present amendment.

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Enclosed is a petition to extend the period for replying for 3 months, to and including February 29, 2000. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: February 28, 2000

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